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Prognostic Indicators of Recurrence of Bacterial Vaginosis

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ABSTRACT Following all forms of therapy for bacterial vaginosis (BV), recurrence rates are extremely high. Many diagnostic tests are available that differentiate bacterial vaginosis from other types of vaginal disorders, but none predict recurrence after treatment, nor are any vetted for monitoring ongoing responses to treatment. Our goal was to determine which tests, and at what optimal times, have prognostic value in predicting recurrence. This prospective cohort study monitored 74 highly recurrent BV patients for up to 9 months. Symptomatic BV patients were treated with oral metronidazole and were evaluated at cessation of treatment and monthly. Index tests included Amsel, Nugent, BV Blue, and Affirm VPIII, as well as a quantitative PCR (qPCR)-based test under initial evaluation here. The qPCR-based LbRC (*Lactobacillus* Relative Composition) assay predicted BV recurrence when performed shortly after oral metronidazole treatment, with both 90% positive predictive values (PPV) and 74% negative predictive values (NPV); the Nugent scores had 93% PPV but poor NPV (57%). No test, at any other visit, was prognostic. The LbRC assay and, to a lesser extent, Nugent tests scored a week after oral metronidiazole predicted recurrence, suggesting that the recurrence in this cohort was predominantly by relapse due to incomplete restoration of eubiosis soon after therapy. This is the first study in an under evaluated population of recurrent BV patients that emphasizes the need for and a pathway to a possible prognostic modality. Given the high recurrence rates of BV, prognostic tests that could influence individualized treatment alternatives are urgently needed.

KEYWORDS Affirm VPIII, Amsel criteria, BV Blue, *Lactobacillus*, Nugent score, bacterial vaginosis, metronidazole

Oral metronidazole is the standard of care for treatment of bacterial vaginosis (BV) (1) because it has broad spectrum activity against anaerobic microbes (2–5), minimal impact on lactobacilli and facultative anaerobes (1, 6), and rarely induces acquired resistance (4). However, all metronidazole regimens result in high recurrence rates of BV, 69 to 80% within 12 months, including patients with relapse or reinfection (7, 8). Recurrence of BV is complex and may result from a variety of mechanisms that differ among individuals (1, 8–13) and has prompted numerous alternatives to metronidazole (14–18). Predicting recurrence in individual patients with acute BV, especially patients with a history of recurrence, has not been addressed and has important implications for customizing acute and long-term maintenance therapies to break the cycle of ongoing recurrence (15).

In the present study, we demonstrate that a novel quantitative PCR (qPCR)-based test, along with LbRC (*Lactobacillus* Relative Composition) and Nugent scores, obtained immediately after oral metronidazole therapy predict whether patients in remission from recurrent BV will sustain that remission or recur later.

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MATERIALS AND METHODS

Patient enrollment. This single crossover prospective trial was performed at a Vaginitis Clinic at Wayne State University in Detroit, MI, from 30 September 2014 to 30 May 2017. Women were enrolled as recurrent BV patients if their patient histories indicated three or more episodes of symptomatic BV in the previous year and had acute symptomatic BV at enrollment. Patients were categorized as BV patients if they were both positive for ≥3 Amsel criteria (vaginal pH ≥ 4.5, positive amine "Whiff" test, >20% clue cells, and grayish-white adherent discharge) (19) and symptomatic with odor, discharge, discomfort, or itching.

A patient was included if she was premenopausal and ≥18 years old; had no mixed infections of vaginal *Candida*, *Trichomonas*, herpes simplex virus, or cervicitis; was willing to refrain from using any other vaginal products during the study period; and was heterosexual and agreed either to use condoms for the duration of the study or to report unprotected sex and to abstain from coitus within 48 h of any study visit and to abstain from alcohol during therapy. She was retained in the study if at any point she became culture positive for *Candida*; if she had a history of vaginal candidiasis she was treated according to the standard of care as defined by Centers for Disease Control and Prevention guidelines. She was excluded from the study if she was pregnant, nursing or at risk of pregnancy; if she was on anticoagulation, lithium, or disulfiram therapies; if she was allergic to metronidazole; if she had used any vaginal antibiotics or antifungals in the previous 10 days; if she would not agree to abstain from vaginal douching or probiotics during the enrolled period; if she required treatment for an abnormal Pap smear or genital cancer; or if she had vaginal bleeding at time of enrollment. Other forms of contraceptives were not exclusionary. Consent and HIPAA forms were explained to each patient, who agreed and signed these forms as a condition for enrollment. The protocol and forms were approved by the Wayne State University Institutional Review Board (IRB 040314M1F). BV patients at enrollment were given the standard of care regimen of oral metronidazole 500 mg twice daily for 7 days.

Reference and index tests. Four vaginal swabs were taken to determine index tests, including Amsel (19) and Nugent (20) scores, two commercial tests, and our qPCR-based test, the LbRC assay. BD Affirm VPIII (Becton, Dickinson, and Co., NJ; performed by the Detroit Medical Center Clinical Laboratory) generates a positive hybridization signal if there are ≥200,000 cells per swab of *G. vaginalis* (21). pH was visually scored using pH paper with a range 3.0 to 5.5 (Hydrion pH test strips). BV Blue (22, 23) (Gryphus Diagnostics, LLC, TN) is an enzymatic assay for sialidase derived from BV-associated species in the swab; the colorimetric assay if positive is blue or green. All tests other than BD Affirm VPIII were performed by clinicians and study personnel.

There is no gold standard for the diagnosis of BV, so as reference standards, we alternatively used Amsel and Nugent scores, and a third more rigorous reference we designate symptomatic BV (sBV), defined as Amsel-positive patients with symptoms that warranted treatment. Clinical information and Amsel scores were not available to the laboratory staff or readers of index tests; conversely, clinicians scoring Amsel criteria had no access to the other test scores, nor did performers or readers of individual tests have access to results of other tests at scoring. Patients returned 7 to 14 days following initiation of oral metronidazole therapy for a follow-up exam to assess outcomes by Amsel and index tests and again once a month to assess long-term outcomes. BD Affirm VPIII and BV Blue tests were repeated only on follow-up, second visits. Patients returned upon request if they experienced symptoms consistent with recurrence. Patients were monitored monthly for 9 months unless they chose to drop out or acquired other exclusion criteria or because of symptomatic recurrence of BV. Recurrent patients were offered either a high-dose vaginal metronidazole treatment or a maintenance regimen from the literature (15), not included in this analysis. All patients also performed daily vaginal self-swabs according to the provided instructions and recorded whether they engaged in sex, with or without a condom, and whether they were in menses. The full protocol is posted online (https://docs.google.com/document/d/1uUZvo0soD2rBCDtMBxsT1TmAJp72CD2E_E6FoWM9Ato/edit?usp=sharing).

Primary end points among BV patients were defined as follows. Bacteriological recurrence was defined as a Nugent score of ≥7. Clinical recurrence, sBV, is defined as an Amsel score of ≥3, including elevated pH, and symptoms presented by the patient, i.e., the need to treat. Remission was defined as the absence of recurrence and considered long-term remission if this persisted ≥3 months after therapy. Refractory was defined as the absence of remission immediately following therapy.

DNA extraction. Vaginal swabs for DNA extraction were submerged in 4 ml of 2-isopropanol 1n 15-ml conical tubes immediately after swabbing. These tubes were stored at room temperature; we determined that cells were not viable and that DNA was not degraded under these conditions for >30 days. Tubes were centrifuged 1,580 × *g* for 30 min, supernatants were discarded, and pellets were drained 15 min. Pellets were resuspended in 300 μl of alkaline lysis buffer (100 mM KOH, 2% Triton X-114, 2 mM EDTA) and heated at 65°C for 2 h. Tubes were centrifuged again (1,580 × *g* for 10 min). Then, 130 μl of supernatant was distributed to pairs of wells in duplicate 96-well sterile polypropylene plates containing 65 μl of neutralizing buffer (200 mM Tris [pH 8.3], 4 mM EDTA, 200 mM HCl). Plates were sealed and stored at −20°C until used for qPCR. The quality of DNA from these preparations was sufficient for qPCR or targeted next-generation sequencing.

qPCR methods. The LbRC assay estimates the relative concentrations of *Lactobacillus* species to the total bacterial titer. It does this by measuring the bacterial titer with a broad-spectrum primer mix in the absence versus presence of blocking oligomers LBB3 and LBB4, which specifically complement *Lactobacillus* species and partially overlap or juxtapose the 3′ end of the broad-spectrum primers, thereby specifically inhibiting amplification of all *Lactobacillus* species by the broad-spectrum primers (24, 25). The assay used in this study differs from the published assay only in that here we used an expanded set of broad-spectrum primers to be more inclusive (see Table S1 in the supplemental material). ΔC_q

TABLE 1 Patient characteristics at enrollment

Characteristic	Data
Total no. of patients	90[a]
Mean (SD)	
No. of visits[b]	5.6 (2.8)
Days in study, range[b]	124 (90), 17–381
pH	5.7 (0.6)
African-American	5.8 (0.3)
Caucasian	5.6 (0.5)
Nugent score	8.6 (1.6)
African-American	8.7 (1.3)
Caucasian	8.4 (1.4)
Amsel score	3.9 (0.4
African-American	3.9 (0.4
Caucasian	3.9 (0.4)
Age (yr), range	34 (7), 21–48
African-American	34 (7)
Caucasian	34 (4)
Race, no. (%)	
African-American	81 (90)
White	7 (8)
Other[c]	1 (1)
Not reporting	1 (1)
Ethnicity, no. (%)	
Non-Hispanic	89 (99)
Hispanic	0 (0)
Not reporting	1 (1)

[a]Includes 16 who were lost to follow-up after enrollment.
[b]Excludes those lost to follow-up.
[c]More than one race.

(quantification cycle [C_q] of *Lactobacillus*-blocked sample $-$ C_q of unblocked sample) measures relative *Lactobacillus* content, given by the formula % *Lactobacillus* $= (100 \cdot \Delta Cq^2)/(\Delta Cq^2 + 1)$, so that a ΔCq score of 0 indicates that a sample has no *Lactobacillus*, 1 indicates that the population was 50% *Lactobacillus*, 2 indicates 80% *Lactobacillus*, etc. (Fig. S1). Here, we incorporated a penalty into the ΔCq score if a sample displayed non-*Lactobacillus* melt profiles to generate the LbRC/5 score, i.e., ΔCq/5. The qPCR conditions are detailed in Table S1 in the supplemental material. Representative qPCR data that generate the LbRC/5 scores from acute, recurring, and remission patient samples illustrate the parameters that influence the score (Fig. S2). Duplicate runs of 235 samples, in which the second run was separated by days to a month from the first, showed excellent agreement of ΔCq and dominant and subdominant melting temperature (T_m) values (Table S2).

Statistical methods. Statistical significance and other analyses followed the STARD guidelines (26), as cited in text, and were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla CA). Categorical data for diagnostic accuracy were analyzed using the Fisher exact test and associated tools to generate 95% confidence intervals. Comparisons between assays in which one assay result was missing were excluded. Significance of comparisons of continuous data were analyzed by *t* tests if the distributions passed the D'Agostino-Pearson omnibus normality test or by the Mann-Whitney test if not ($P \leq 0.05$ was considered significant).

RESULTS

Patient profiles. The study enrolled 90 recurrent BV patients for a mean of 5.6 monthly visits, ranging from 2 to 11 visits, and excluded 16 from outcome classification because they did not return after their initial visit (Table 1). BV patient mean scores at enrollment were pH 5.7, Nugent 8.6, and Amsel 3.9. The majority of BV patients were African-American (90%) compared to 8% Caucasian, but these groups were not significantly different in mean age, pH, and Nugent or Amsel scores at enrollment (Wilcoxon matched-pair signed-rank tests). Patients lost to follow-up were not a biased subset of the total cohort.

Response to treatment. After standard-of care-oral metronidazole (SOC) therapy among 74 patients, 46 (62%) achieved at least short-term remission, and 28 (38%) were refractory at the first follow-up visit. Among the 46 patients in initial remission, 28 (60%) recurred at a subsequent visit, i.e., 38% of the total tracked cohort. Thus, overall

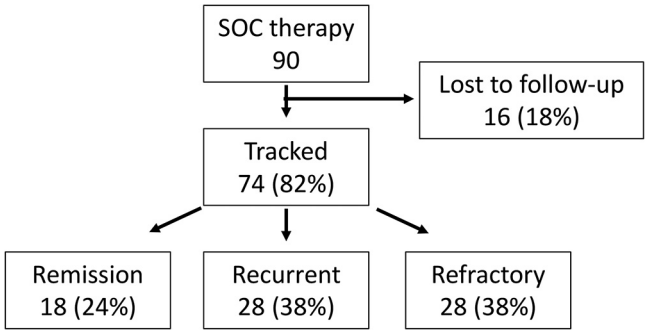

FIG 1 Flow diagram of enrollment and responses to therapy. Only patients with at least three visits in remission after a treatment were scored as remission, excluding patients who dropped from the study before this while in remission. Recurrent, at least one posttreatment visit in remission before recurrence; refractory, symptomatic BV at first posttreatment visit.

response to metronidazole was poor; 56 of the 74 patients (76%) who were not lost to follow-up recurred or had a refractory response (Fig. 1). The percentage of patients that achieved long-term remission after SOC therapy (24%) was unexpectedly high, given this cohort's history of recurrence. The numbers of remission versus recurrent versus refractory outcomes were not significantly different ($P = 0.269$), i.e., an individual patient was approximately equally likely to respond in any of the three ways.

Diagnostic performance of BV tests. Although the purpose of the study was to evaluate the prognostic abilities of diagnostic tests for BV, we first evaluated their ability to correctly diagnose BV. We alternatively used Amsel, Nugent, or sBV as reference standards, as well as each of these plus BV Blue, Affirm, pH alone, and the LbRC scores as index tests.

Accuracies of index tests of all pooled samples relative to three reference standards (Amsel, sBV, and Nugent) were compared (Fig. 2). For this analysis, Nugent scores 7 to 10 were categorized as positive, and Nugent scores 0 to 6 were categorized as negative. The three reference tests were approximately equivalent for most index tests, whose accuracy varied by <5%. Amsel, pH, and Nugent scores were most accurate, ranging from 84 to 94% accurate to their reference tests. LbRC/5 at an optimal threshold score

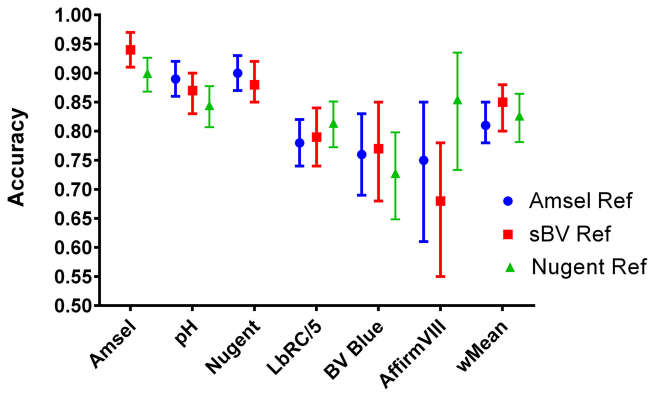

FIG 2 Accuracies of the index tests (*x* axis) relative to reference tests Amsel, sBV (symptomatic, Amsel positive), and Nugent score. The Nugent score breakpoint as a reference test was defined as not BV if 0 to 6 and as BV if 7 to 10. Several breakpoints of Nugent scores were considered as index tests (#/#); the first number is the upper limit considered not BV, and the second number is the lower limit considered BV. LbRC/5 scoring is described in the text; threshold numbers following these are the maximum scores defined as BV. wMean is the mean of all index scores per reference test, weighted by the number of samples in each index test. Error bars indicat 95% CI. *, Significantly different from the optimal index score per reference test; those using Amsel as the reference are compared to the Nugent 6/7 test, and those using sBV or Nugent 6/7 as the reference tests are compared to the Amsel index tests. Comparisons were made using the Fisher exact test ($P < 0.05$). Data for all patients at all visits were pooled for this analysis.

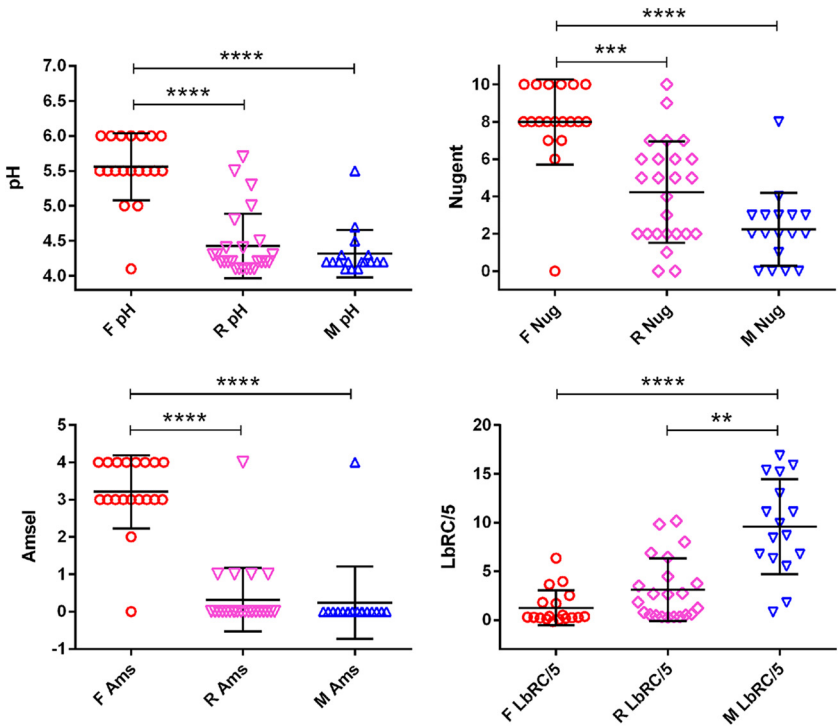

FIG 3 Distribution of immediate posttreatment visit scores grouped by patient outcome defined as follows: F, refractory, i.e., Amsel positive and symptomatic requiring treatment, at the posttreatment visit; R, recurrent if the patient was in remission posttreatment but became Amsel positive and needed treatment at some later visit; M, remission if the patient did not become Amsel positive or need treatment at any subsequent visit. Markers denote individual patients; means and standard deviations are shown as bars. Comparisons that showed significant differences are indicated by asterisks. Tests were evaluated by Kruskal-Wallis analysis, followed by Dunn's multiple-comparison test.

of 1 ranged from 78 to 82% accurate, significantly lower than Amsel, pH, or Nugent scores. BV Blue and Affirm VPIII tests were less accurate than the other standard tests (Fig. 2).

Prognostic value of BV diagnostic tests at the posttreatment visit. Because recurrence rates among BV patients are so high, we sought to determine whether any of the diagnostic tests could predict subsequent recurrence of patients who initially achieved remission following SOC therapy. For patient management, we defined recurrence as either Amsel positive or Amsel positive plus symptoms requiring treatment (sBV). Distributions of scores of individual patients at this visit (Fig. 3) showed that only LbRC/5 scores differed significantly between patients in remission who would later recur (R patients) versus those who would remain in remission (M patients). At this visit, the LbRC/5 scores of patients in remission but destined to recur later were not significantly different from the scores of patients who were refractory.

We adapted diagnostic statistics to this prognostic application; in this context a true positive test means that a positive score of a remission patient at the posttreatment visit (LbRC/5 $<$ 5 or Nugent $\geq$ 4) corresponds to recurrence at a later visit and, conversely, that a true negative means that a negative score of a remission patient at the posttreatment visit (LbRC/5 $\geq$ 5 or Nugent $<$ 4) corresponds to long-term remission (remission for at least three subsequent visits or to the end of enrollment, whichever is longer). It follows in this context that strong positive predictive values (PPV) mean a positive score of a patient in remission at posttreatment strongly predicts later recurrence and that strong negative predictive values (NPV) strongly predict long-term remission for a patient with a negative posttreatment score. Only the LbRC/5 and Nugent tests had significantly different distributions of scores for predicting continued remission versus later recurrence ($P < 0.05$; Table 2). LbRC/5 using an optimal break-

TABLE 2 Prognostic performances of LbRC/5, Nugent, and pH at the posttreatment visit[a]

	LbRC/5 (5)		Nugent (4)		
Parameter	sBV	Amsel	sBV	Amsel	pH (4.5)
P	0.0002	0.007	0.002	0.017	0.079
Odds ratio	23.8	6.0	17.3	5.3	0.3
95% CI	3.99–142	1.6–22	1.98–151	1.41–20	0.09–0.98
Sensitivity	0.77	0.67	0.52	0.57	0.24
95% CI	0.55–0.92	0.45–0.84	0.31–0.72	0.37–0.76	0.09–0.45
Specificity	0.88	0.75	0.94	0.80	0.48
95% CI	0.62–0.98	0.51–0.91	0.71–1.0	0.56–0.94	0.28–0.69
PPV	0.90	0.76	0.93	0.80	0.32
95% CI	0.67–0.99	0.53–0.92	0.66–1.0	0.56–0.94	0.13–0.57
NPV	0.74	0.65	0.57	0.57	0.39
95% CI	0.49–0.91	0.43–0.84	0.37–0.76	0.37–0.76	0.22–0.58
LR	6.2	2.7	8.8	2.9	0.5

[a]Asymptomatic patients at the posttreatment visit were grouped by whether they remained in remission (17 patients) or recurred at some later visit (25 patients). Recurrence was defined either as sBV (Amsel positive requiring treatment) or as Amsel positive. Tests were scored as positive or negative if they fell above or below the breakpoints indicated in parentheses. Amsel, BV Blue, and Affirm VPIII tests were also analyzed, but the results are not shown because the scores were distributed randomly with respect to outcome ($P > 0.19$). 95% CI, 95% confidence interval; LR, likelihood ratio.

point of 5 had strong PPV (0.90) and useful NPV (0.74). Nugent scoring using an optimal Nugent breakpoint of 3 to 4 had excellent PPV (0.93) but poor NPV (0.57). Similar patterns were seen when only Amsel criteria were used to define later recurrence, but tests had lower PPV relative to the sBV criteria. Breakpoints were optimized based on distributions of scores in remission versus recurrent groups and on receiver operating characteristic (ROC) analyses (Fig. 3 and Fig. S3). Vaginal pH generated nearly significantly different distributions of remission versus recurrent outcomes ($P = 0.079$) but had no predictive value.

Other than at the immediate posttreatment visit, no test was prognostic of clinical outcome at any other visit, including the initial, pretreatment, symptomatic visit. Since it is reasonable to suspect that vaginal bacterial compositions begin to shift in advance of symptomatic BV, we specifically sought to determine whether scores from visits just preceding recurrence visits were predictive of those recurrences. For 21 patients for which data were available, the test scores for each patient at the visit preceding the recurrence visit were compared to the mean scores of the same patient for all other remission visits. Surprisingly, the test scores at the prerecurrence visits were not significantly different from the mean of other visits in remission ($P \geq 0.099$, Table 3).

Collectively, these data indicate that at no specific point in time, other than immediately after treatment, does an individual's status, as defined by any of the

TABLE 3 Diagnostic test scores at the remission visits of recurrent and remission patients[a]

	Test score (SD) or *P* value			
Category	pH	Amsel	Nugent	LbRC/5
Prerecurrent only	4.5 (0.5)	0.6 (1.2)	4.0 (3.0)	5.2 (5.9)
Not prerecurrent*	4.7 (0.6)	0.9 (1.3)	4.4 (2.6)	3.1 (3.5)
Prerecurrent vs not prerecurrent (*P*)	0.276	0.102	0.277	0.099
Remission†	4.4 (0.3)	0.3 (0.4)	2.0 (1.4)	8.1 (3.3)
Not prerecurrent vs remission (*P*)	0.251	0.564	0.002‡	<0.0001‡

[a]Results are presented as means (with standard deviations in parentheses) except where data are indicated as *P* values. Comparisons were averaged across the visits of 21 recurrent patients when in remission, excluding the prerecurrent visit (*), or across all remission visits of 17 remission patients (†). *P* values were calculated by the Wilcoxon matched-pair signed-rank test, except where distributions were normal as noted (‡), where they were calculated using unpaired *t* tests.

diagnostic tests, predict later recurrence. Despite the lack of prognostic value of visits other than posttreatment, patients who eventually recur had higher mean Nugent scores and lower mean LbRC/5 scores while in remission than did patients who remained in remission (Table 3). This reflects more instability in these scores over time in the recurrent group.

DISCUSSION

The problems faced by the clinician in managing BV patients are refractory responses and recurrence at high rates following SOC therapy. Conventional tests for diagnosing BV are accurate, not excellent, both historically and in this study, but neither these nor molecular tests have been vetted for their ability to predict which patients will be refractory and which will respond initially but later recur. Having this information when the patient first presents, or at the posttreatment visit, would enable the clinician to tailor alternative treatments as needed to reduce the probability of these undesirable outcomes.

The most significant finding of this study was that the microbial composition of bacterial vaginosis patients within 7 days of finishing conventional oral metronidazole therapy was pivotal in whether they would remain in remission versus recur. This status was best quantified by the qPCR-based assay, LbRC/5. Based on the threshold of prognostic LbRC/5, asymptomatic patients in remission who host any detectable species other than *Lactobacillus*, i.e., BV-associated species, immediately after treatment have a 90% chance of recurring (95% confidence interval [CI] = 67 to 99%). Conversely, patients in remission immediately after treatment for which *Lactobacillus* is >96% dominant (LbRC/5 $\geq$ 5) have a 74% chance of remaining in remission (95% CI = 67 to 99%). A Nugent score of $\geq$4 at this early visit also predicts recurrence but has poor negative predictive value; a patient with a Nugent score of 0 to 3 has only a 57% chance of staying in remission. If these associations hold in subsequent larger studies, at least among recurrent, predominantly African-American cohorts, they will warrant a trial to apply extended or alternative treatments, including probiotics, to patients at posttreatment visits in remission, based on poor LbRC/5 or Nugent scores.

We saw a lack of association of any diagnostic test score with clinical outcome at the initial pretreatment visit, the visit just preceding recurrence, or at any posttreatment visit other than immediately after treatment. This suggests that the initial vaginal composition or status of the pretreated or the prerecurrent patient, at least as measured by these tests, is not a key determinant of clinical outcome. If there are pivotal bacterial determinants at these times, they will likely derive from species or strain level differences that assays currently in clinical use cannot distinguish. Three studies have addressed this issue using qPCR (27, 28) or next-generation sequencing (29) approaches and were limited by short-term follow-up $\leq$46 days posttreatment, which probably clustered patients that would have recurred into the nonrecurrent groups. No species was found to be associated with recurrence pre- or posttreatment in all three studies, and the associations that were found in individual studies were insufficiently strong to be prognostic. These mixed results and our data together suggest that it is less important which of the BV-associated species is found, only that whatever they are, conventional therapy reduces them to or below the limit of detection of a qPCR assay.

The overall response of our cohort of recurrent patients to SOC therapy was poor in that 76% of tracked patients either recurred or were refractory. Despite a history of frequent and recent recurrences, 18% of patients achieved long-term remission. Several explanations may account for this response. Some of these women may have been recurrent due to reinfection via coitus and had changed partners before or early in the study. Some may have been misdiagnosed prior to the study and had other types of vaginitis and thus only had a single episode of BV at enrollment. Some may have been one treatment away from long-term remission, i.e., each successive treatment moved them incrementally to a more stable vaginal microbiome. The tests applied to the cohort could not distinguish this subgroup from the others at their initial symptomatic visit, so by these criteria they did not have a less severe form of BV. Pending next-

generation sequencing studies may show whether there were differences in composition of this subgroup that associates with their long-term remission.

The LbRC test is essentially a molecular Nugent test, in that it measures the relative abundance of *Lactobacillus* and reports whether even traces of non-*Lactobacillus* species are detected. While it is adequate at diagnosing BV, it suffers from false-positive scores because it is too sensitive to dysbiosis. The empirically optimized threshold score for diagnosing BV was ≤1, approximately 50% or less *Lactobacillus* species; the test is not sufficiently precise to resolve scores under 1, where the likely true diagnostic differentiation lies. This disadvantage turns into an advantage in prognosis because the optimized prognostic threshold LbRC score was ≥5, or approximately 95% *Lactobacillus*. This explains why Nugent scores 0 to 3 did not predict remission at the posttreatment visit even though Nugent scores 4 to 10 predicted recurrence. The bimodal distribution of Nugent scores among patients who will later recur indicates that nearly half of these patients have low Nugent scores 0 to 3 who nevertheless have LbRC/5 scores below 5. These low Nugent scores were not prognostic because they do not distinguish patients whose levels of *Lactobacillus* were in the 80 to 95% range from patients with levels of *Lactobacillus* above 95%, i.e., those less likely to recur because they have very few non-*Lactobacillus* species.

Our observation that only the posttreatment visit has this prognostic information is logical at least in hindsight and tells a story about the pathobiology of BV. Primarily, it means that eradication of non-*Lactobacillus*, BV-associated species by SOC therapy to nearly undetectable levels is crucial to guarantee long-term remission. Second, the lack of prognostic signatures at other remission visits means that the battle for dominance among vaginal species is an ongoing process which at no specific single time is decisive, until recurrence. Getting off to a good start, posttherapy, is the only path to success long term. Finally, the unique association of this visit with long-term outcome suggests that eventual recurrence was not driven by events unrelated to therapy. If coitus or reinfection, for example, were driving recurrence in this cohort, the association of response to therapy and long-term outcome would have blurred, and prognostic indicators would not have been found.

Interpretations of findings in this study are to be extrapolated cautiously because of its limitations. These include that our cohort was predominantly African-American, we had high drop rates after therapy, coitus was self-reported, and data on the numbers of partners were not collected. Nevertheless, our prognostic findings clearly warrant larger studies on more diverse groups. If validated, clinical extensions of these findings may be to apply extended, higher-dose, or combination therapies that include probiotics, specifically to patients in remission at the post-SOC treatment visit if they are at risk of recurrence by the LbRC or even the Nugent score. Preemptive therapy is not standard of care for BV, but it has been applied successfully, for example, as twice-weekly regimens of high-dose vaginal metronidazole/miconazole (15). Patients do well during the treatment regimen but recur after therapy ends. We suggest that the secondary treatment should occur at the posttreatment visit for only patients that score as high risk for recurrence, before the non-*Lactobacillus* species that are still present have the opportunity to retake the vaginal battleground.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00227-19.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

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